EFFECTS OF CHRONIC EXCESS SALT FEEDING

Elevation of Plasma Cholesterol in Rats and Dogs*

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The interrelationship of hypertension and atherosclerosis has been the subject of repeated investigation and a voluminous literature. At the present time it is generally thought that there is no basic etiological relationship between the two diseases; however, it appears that hypertension accelerates the progress of established atherosclerosis and that the inception of atherosclerosis is hastened by the presence of antecedent hypertension (1).

For some years we have been studying the effects of chronic excess salt feeding in man and animals in an effort to explore the etiologic relationship between salt ingestion and human essential hypertension (2–5). We recently concluded such a study on dogs and rats. The results suggest that chronic salt ingestion is capable of elevating the concentration of plasma lipids. The available evidence is insufficient to conclude that such a factor operates in man to raise plasma lipid levels or that it is involved in the production of atherosclerosis, either human or experimental. Nonetheless, since salt has been invoked in the pathogenesis of human hypertension (4), the present studies suggest one possible biochemical basis for a link between atherosclerosis and hypertension.

EXPERIMENTAL

Animals and Feeding Programs:

1. Rats.—All animals were Sprague-Dawley females.

Group I consisted of 16 animals of which 11 were salt-fed and 5 were controls; salt feeding was begun at age 2½ months and continued up to the time of sacrifice, more than 15 months later. Group II included 41 animals, 11 controls and 15 on each of the two salt regimens described below; salt feeding was started at age 1 month and blood for cholesterols was obtained 6½ months later. Group III with 36 animals, of which 12 were controls, was similar to Group II except that salt feeding began at 3 weeks of age and blood for cholesterols was obtained 8 weeks later. All animals were housed in air-conditioned rooms, 2 to 6 animals per cage, and were allowed free access to drinking (tap) water and food. Repeated analyses of the tap water showed it to contain only 0.5 to 0.7 m.eq. of sodium per liter. The basic diet that all control animals received in unmodified form was Ralston Purina fox chow pellets, which by our analyses contained sodium in amounts ranging from 0.51 to 0.74 per cent calculated as NaCl.

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Test animals received the same chow to which one of two supplementary sodium-containing salts had been added by the manufacturer so that the final concentration calculated as NaCl approximated 8 per cent by weight. In one food ("8 per cent NaCl food") the supplementary salt was sodium chloride: our analyses indicated an average of 8.09 per cent NaCl.

The other food ("sea-salt food") had 11.6 per cent "sea-salt" added, an amount which was calculated to give a sodium concentration equal to that in the 8 per cent NaCl food; our analyses showed this food to have less than calculated, namely an average of 7.28 per cent (as NaCl). The rationale of using sea-salt for inducing hypertension is not pertinent to the current studies and therefore discussion of its use will be deferred to another report. Information from the producers of this special salt[1] indicate that it is obtained by evaporating sea water and is finally dried at an air temperature of 1100°F. All rats were weighed monthly when blood pressures were measured and equivalent food consumption among the various groups was indicated by: (*a*) similar weight and growth curves, and (*b*) at intervals the food actually consumed was carefully checked by weighing food dishes daily over 4-day periods.

2. Dogs.—Care and housing: The colony consisted of 17 pure bred beagles, 16 females and one castrated male. The original colony had 16 animals born between November 16, 1955, and February 7, 1956, purchased as weanling females but found to contain one male by error. It was elected to keep this male and castrate him when it became technically feasible; through oversight this was delayed until the age of 9 months by which time impregnation of one female (No. 8-5—control) had occurred. This female delivered 2 normal pups on October 13, 1956, of which a female (No. 3-24—control) was added to the experimental group. At the beginning of the salt feeding program animals were distributed by weight as uniformly as possible among the 4 groups. The animals were housed in large screened pens with cement floors in a heated building, by groups according to the salt feeding regimen. These pens were scrubbed and cleaned thoroughly every day. All dogs were put outside in special runs for several hours each day except in the most inclement weather. Their care, handling, and health were supervised by a staff veterinarian and each animal received the usual routine immunizations and semiannual deworming procedures. On this regimen they remained uniformly healthy and active.

Feeding: All beagles received the same basic diet of kibble, canned horse meat, a sodium-free mineral-vitamin supplement, and water. During the first months of the study when they were still young, animals were fed 3 times a day and received milk; the frequency of feeding as well as the amount of milk were gradually decreased and after 6 months of age they were fed only once a day without milk. Each animal had its own food dish but members of a group were fed simultaneously; feedings were supervised by an animal attendant to prevent a dog from eating food other than its own and in the occasional cases where this occurred animals were fed separately as long as necessary to prevent it. The stable weights suggested that food consumption was at a constant level.

Each animal daily was fed and consumed an average of 200 gm. kibble, 100 gm. canned horse meat, and 1 teaspoon of the mineral-vitamin supplement. The 5 *control* animals received this diet, plus tap water *ad lib.* The 4 "1 per cent NaCl-H_2O" animals were fed this diet but drank 1 per cent saline in place of tap water, consuming an average of about 0.9 liter per dog per day. The 4 "2 per cent NaCl food" animals were fed kibble calculated to contain 2 per cent NaCl, and otherwise as the control animals. The 4 "6 per cent NaCl food" animals differed from the preceding group only in that their kibble contained 6 per cent NaCl. Analysis of the various foods for sodium concentration in the author's laboratory gave the following average concentrations, calculated as NaCl: unmodified kibble 1.00 per cent; 2 per cent NaCl kibble, 2.46 per cent; 6 per cent NaCl kibble, 5.97 per cent; canned horse meat, 0.48 per cent. The

[1] The author is indebted to the Trace Elements Corporation, Houston, for a supply of admiral brand trace element sea salt sufficient to initiate these experiments.

daily average NaCl intake for the groups was estimated to have been as follows: Control, 2.5 gm.; 1 per cent NaCl-H_2O, 11.5 gm.; 2 per cent NaCl food, 5.5 gm.; 6 per cent NaCl food, 12.5 gm. The 3 groups on extra salt began receiving it when the members ranged from 2½ to 4½ months of age and at the time of the latest blood lipid and blood pressure measurements had completed from 45 to 48 months on this regimen. The 4 animals in the 6 per cent NaCl food group, received 2 per cent NaCl food during the first 7½ months of the experiment, after which they received the 6 per cent NaCl food continuously.

Blood Pressure Measurements:

1. Rats.—The microphonic method of Friedman and Freed (6) was used; measurements were made under light ether anesthesia, using a flowing oxygen-ether mixture, in a special box with temperature thermostatically controlled at 38°C. At least 4 systolic readings were recorded with each measurement and the average of these 4 was used. This technique has given highly reproducible readings for several years and many successive monthly measurements on control animals up to 21 months of age have never shown systolic pressures consistently in excess of 130 mm. Hg. For this reason, animals with pressures of 140 mm. Hg or above have been considered hypertensive. Blood pressure measurements were made at monthly intervals except among the animals of Group III, on which measurements were made only once, just prior to the bleeding for plasma cholesterol concentrations after 8 weeks of salt feeding.

2. Dogs.—Measurements were made in one of two ways on unanesthetized animals; (*a*) direct, intra-arterial femoral mean pressures were recorded using a Tycos aneroid manometer (calibrated against a mercury manometer with each use); (*b*) indirect, by means of the Friedman and Freed technique (6) with which accurate systolic pressures were recorded easily. Since this latter method is simple, appears to be accurate, requires little training of animals, and has not been described for dogs, a description of and justification for its use are presented briefly.

The technique for dogs is basically similar to the original description for rats and only the pertinent modifications are noted. The tail is clipped closely for 6-to 8 inches from the base towards the tip, after which a depilatory of 20 per cent Na_2S in water is applied for several minutes to the entire circumference of the tail over an area 2 to 3 inches in length beginning about 3 inches from the base of the tail; ordinarily at the end of this time the stubble has turned yellow and soft and is easily wiped off after which the area is thoroughly rinsed with 70 per cent alcohol. To the area which is now smooth and clean of hair a small amount of vaseline is applied where the microphonic attachment is placed—generally best in a lateral position. The signal to noise ratio is significantly increased by the foregoing procedures, and the pulse sounds usually come through clearly. A small rubber cuff 2 inches wide, made up like a standard cuff used for measuring human blood pressure is applied above the microphone near the base of the tail and connected to a mercury manometer and bulb. Systolic pressure is recorded as for the rat tail technique. Interestingly, in some animals, a diastolic muffling occurs without disappearance of the sound. It is important that the animals be warm and comfortable. Therefore, we ordinarily measure pressures in a room temperature of about 27–30°C., and do not allow the animals outdoors beforehand because of possible cooling with constriction of the tail vessels. A thick foam rubber mattress beneath the animals increased their comfort with resulting greater relaxation. As judged by the pulse rate, most animals are not excited by this procedure, although the usual precautions concerning noise, motion, strangers, etc., must be observed. Results obtained with this technique were compared with those obtained by simultaneous direct iliac artery cannulation in two anesthetized dogs using a Sanborn transducer and multichannel recorder. Various manipulations of blood pressure including intravenous epinephrine indicated remarkably good correlation in systolic readings,

and in one animal in which diastolic muffling occurred the values for diastolic pressure were almost identical with those obtained using the direct technique. In one animal, systolic pressure by the tail technique ranged from 0 to 6 mm. Hg higher than direct iliac pressures; in the other dog the indirect systolic pressures were recorded −1 to +20 mm. different from the iliac pressures, with an average of +11 mm. Hg. It was thought that the slightly higher pressures recorded with the tail technique were due to the sensitivity of the microphone resulting in faint pulse sounds prior to true systolic pressures. By using as routine direct and indirect methods simultaneously, it is likely that the small differences recorded could be decreased by learning the point at which these early sounds of the tail pulse become significant.

TABLE I

Group I. Female Rats, 18 Months of Age at Sacrifice

Rat	Food	Mos. on diet	Body weight	Systolic B.P.	BUN	Total cholesterol
			gm.	*mm. Hg*	*mg. per cent*	*mg. per cent*
427	Control	17½	306	123	N.D.	95
429	"	"	302	109	9.1	95
430	"	"	342	125	10.2	75
433	"	"	270	96	N.D.	92
435	"	"	312	109	19.4	106
Average (and S.D.)			306.4 (±25.7)	112.4 (±11.9)	12.9	92.6 (±11.2)
401	11.6 per cent sea salt	15½	268	160***	11.1	121**
402	" " " " "	"	326	177***	N.D.	193***
403	" " " " "	"	235**	157***	12.7	400***
407	" " " " "	"	252**	125	N.D.	115**
410	" " " " "	"	314	159***	10.3	160***
411	" " " " "	"	272	130	N.D.	198***
412	" " " " "	"	236**	166***	10.8	100
413	" " " " "	"	267	144**	10.3	210***
414	" " " " "	"	332	133	N.D.	125**
417	" " " " "	"	223***	162***	8.6	441***
424	" " " " "	"	243**	151***	17.2	380***
Average (and S.D.)			269.8 (±38.2)	151.3 (±16.4)	11.6 (±2.8)	222.1 (±74.8)

Systolic blood pressures are the average of last 3 values, obtained at monthly intervals.
BUN, blood urea nitrogen.
N.D., not done.
** and ***, differs from mean value in control groups by 2 or 3 S.D., respectively. Salt-fed group had significantly ($p < 0.01$) higher mean systolic blood pressure and plasma cholesterol than control group.

We have not attempted this refinement but in view of the correspondence between the direct and indirect methods, we have adopted the latter for routine measurements and the blood pressures recorded in this paper are those obtained by the tail method. When it became apparent that salt feeding was not resulting in significant elevation of blood pressure, measurements were recorded at irregular intervals. The values used in this report are those obtained during the month in which blood was removed for estimation of cholesterol concentration, and agree with earlier values, within expected limits of variation. About 10 to 15 readings were made on each animal during a single measurement and the final 4 values were averaged. Variations of pressure among these 4 readings were insignificant.

Miscellaneous Methods:

Blood was obtained from rats by nicking the tail and bleeding directly into heparinized tubes; dogs were bled from an extremity by venepuncture, and the blood put into heparinized

tubes. Bloods were centrifuged immediately, plasma removed and refrigerated during the 1 to 2 hours before extraction of cholesterol was carried out.

Cholesterol was measured by the method of Abell, Levy, Brodie, and Kendall (7) and blood urea nitrogen by Conway's microdiffusion technique (8); statistical significance of the difference between the means of groups was calculated by Student's t test using the modern tables of Fisher and Yates (9); p values of <0.05 were required for differences to be considered significant, and use of the phrase "significant difference" in this paper implies $p < 0.05$.

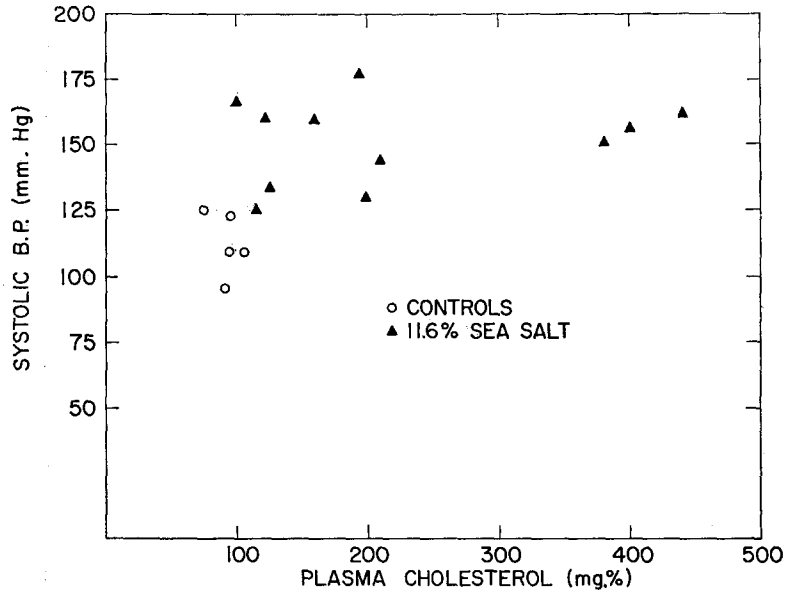

FIG. 1. Effect of chronic salt feeding on blood pressure and plasma cholesterol levels in female rats. Results after 15½ months.

RESULTS

Rats.—Group I (Table I). The salt-fed animals in this group had been on supplemental salt for over 15 months when blood was obtained for cholesterol estimation. Both mean systolic blood pressure and plasma cholesterol were higher in the salt-fed than in the control group. However, among individual members fed salt, the correlation between an increase in cholesterol and in blood pressure was not constant or direct. For instance rats 412 and 417 with similar pressures of 166 and 162 mm. Hg, respectively, had, in the former case a plasma cholesterol of 100 mg. per cent, and in the latter instance 441 mg. per cent. Similarly rat 401 had a pressure of 160 mm. Hg with a plasma cholesterol of 121 mg. per cent while No. 403 with a pressure of 157 mm. Hg had a plasma cholesterol of 400 mg. per cent. Inspection of the table indicates other, if lesser, disparities. The data are presented graphically in Fig. 1 which emphasizes that while there is a trend toward higher blood pressures being associated

TABLE II

Group II. Female Rats, 7½ Months of Age at Time of Bleeding for Plasma Cholesterol

Rat	Food	Mos. on diet	Body weight	Systolic B. P.	Total cholesterol
			gm.	*mm. Hg*	*mg. per cent*
557	Control	7	297	114	113
558	"	"	297	124	63
559	"	"	232	124	81
561	"	"	268	126	81
562	"	"	242	120	99
563	"	"	269	126	125
564	"	"	255	123	113
565	"	"	292	132	90
566	"	"	263	124	89
567	"	"	260	137	63
568	"	"	285	138	94
Average (and S.D.)			269.1 (±21.8)	126.2 (±7.1)	91.9 (±19.9)
501	11.6 per cent sea salt	6½	262	154***	125
502	" " " " "	"	249	122	138**
508	" " " " "	"	263	180***	125
511	" " " " "	"	239	120	119
514	" " " " "	"	283	166***	113
518	" " " " "	"	249	172***	71
520	" " " " "	"	281	162***	123
522	" " " " "	"	245	182***	132**
523	" " " " "	"	263	136	106
524	" " " " "	"	275	162***	123
525	" " " " "	"	265	170***	113
526	" " " " "	"	239	135	85
527	" " " " "	"	271	194***	143**
531	" " " " "	"	264	120	96
532	" " " " "	"	256	130	125
Average (and S.D.)			260.3 (±14.0)	153.7 (±24.6)	115.8 (±19.5
533	8 per cent NaCl	6½	277	140**	109
534	" " " "	"	270	190***	155***
535	" " " "	"	254	145**	90
536	" " " "	"	270	148***	106
537	" " " "	"	258	138	113
538	" " " "	"	263	142**	90
540	" " " "	"	253	150***	104
541	" " " "	"	271	138	113
546	" " " "	"	247	124	99

TABLE II (*continued*)

Rat	Food	Mos. on diet	Body weight	Systolic B. P.	Total cholesterol
			gm.	*mm. Hg*	*mg. per cent*
547	8 per cent Na Cl	6½	268	116	73
549	" " " "	"	268	134	106
551	" " " "	"	222**	126	90
552	" " " "	"	245	126	99
554	" " " "	"	267	132	81
555	" " " "	"	225**	153***	99
Average (and S.D.)			257.2 (±16.5)	140.1 (±17.3)	101.8 (±18.6)

Systolic blood pressures are those for the last month of the experiment.

** and ***, differs from mean value in control group by 2 or 3 S.D., respectively. Both salt fed groups had higher mean B.P. (sea salt < 0.01, NaCl $< 0.05 > 0.01$) than control group. Sea salt group only had higher ($p < 0.01$) mean plasma cholesterol than control group.

with higher cholesterols, there are significant exceptions. Neither blood pressure nor cholesterol concentration was correlated with weight.

Group II (Table II). Supplemental salt had been supplied to the test animals in this group for 6½ months at the time of the plasma cholesterol and blood pressure measurements. Both salt-fed groups had mean blood pressure elevations compared with the control group (the sea salt group being higher) but only the group fed sea salt had a significant increase in cholesterol. Among individual salt-fed animals in this group elevations in blood pressure were common but elevations in cholesterol were not. Among the 4 rats with significant elevation in plasma cholesterol (Nos. 502, 522, 527, 534) 3 had significant elevations in blood pressure. By contrast, among the 16 animals with elevated blood pressure, there were only 3 with increased plasma cholesterol. These data could be interpreted to indicate that (*a*) if an elevated blood pressure *per se* will *cause* an elevation in plasma cholesterol, the pressure must exist for a longer period than was present here; or (*b*) both the elevated pressure and increase in cholesterol were the result of a common stimulus but the pressure had no causal relationship to the cholesterol levels; or (*c*) the few, and modest, elevations in plasma cholesterol found here were entirely fortuitous. Body weight again showed no correlation with either blood pressure or cholesterol concentration. See Figure 2.

Group III (Table III). The salt-fed animals in this group had been on salt food for only 2 months. Both series fed added salt had higher mean blood pressures and plasma cholesterol concentrations than their controls (Table III). Individual significant elevations in cholesterol concentration were present in only 4 animals, all of which had elevated pressures as well; among the 24 salt-fed animals, 13 had elevated pressures (as did 1 control). Comparison of

the mean blood pressures in animals fed the same foods in groups II and III were not different; however, all 3 series in the older animals of group II had significantly higher plasma cholesterol concentrations: calculation of values for t gave 4.40 ($p \ll 0.01$), 2.85 ($p < 0.01$) and 2.54 ($p < 0.05 > 0.01$) as estimates for significance of the difference between the mean cholesterol concentrations in the sea salt, NaCl, and controls in these 2 groups, respectively.

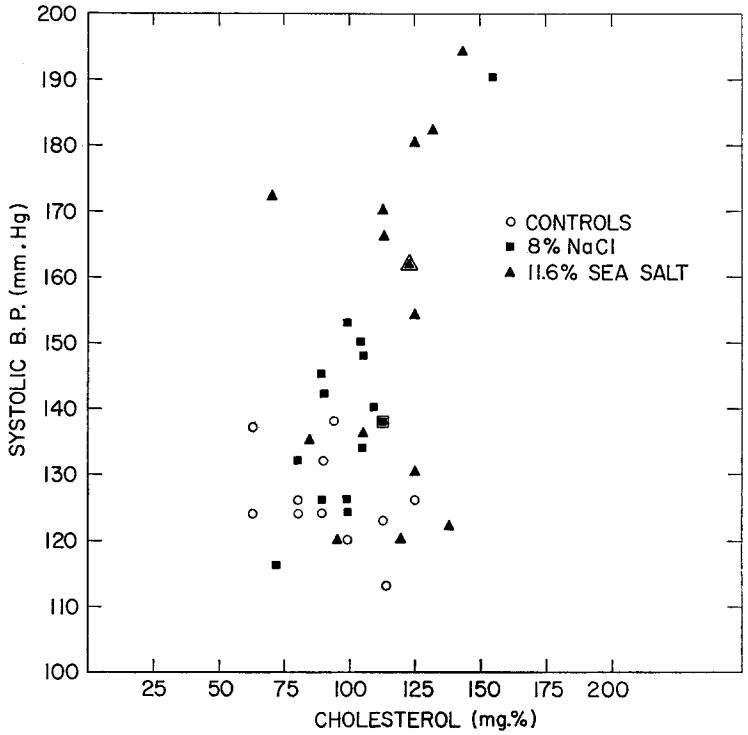

FIG. 2. Effect of chronic salt feeding on blood pressure and plasma cholesterol levels in female rats. Results after 6½ months.

Since the control animals of group II at 7½ months of age, had increased plasma cholesterol concentration compared with the controls in group III at 3 months, some of the elevation in plasma cholesterol found in the animals fed salt for 6½ months must be ascribed to aging. However, it is doubtful that aging alone is a cause of the significant increase in plasma cholesterol levels found in this study for control animals in groups I and II, had similar plasma cholesterol levels at 17½ and 7½ months of age, respectively. No correlations could be established with weight. See Figure 3.

Dogs.—(Table IV, Fig. 4). The control and salt-fed groups did not differ

TABLE III

Group III. Female Rats, 11 Weeks of Age When Blood for Plasma Cholesterol Obtained and Systolic Blood Pressures Measured

Rat	Food	Mos. on diet	Body weight	Systolic B. P.	BUN	Total cholesterol
			gm.	*mm. Hg*	*mg. per cent*	*mg. per cent*
765	Control	2	191	122	18.7	60
766	"	2	225	126	17.4	74
767	"	2	204	125	29.7	85
768	"	2	210	126	20.9	74
769	"	2	200	130	19.6	81
770	"	2	215	142**	18.6	90
773	"	2	209	129	N.D.	81
774	"	2	222	132	"	50
775	"	2	223	120	"	74
776	"	2	225	134	"	85
777	"	2	235	132	"	81
778	"	2	240	134	"	60
Average (and S.D.)			216.6 (±14.4)	129.3 (±6.0)	20.8 (±4.5)	74.6 (± 12.1)
701	11.6 per cent sea salt	2	210	194***	13.0	85
702	" " " " "	2	181**	133	21.6	81
703	" " " " "	2	218	134	18.4	94
704	" " " " "	2	201	152***	20.9	85
705	" " " " "	2	214	182***	23.4	100**
706	" " " " "	2	215	154***	17.8	90
709	" " " " "	2	189	122	N.D.	85
710	" " " " "	2	211	174***	"	100**
711	" " " " "	2	205	136	"	81
712	" " " " "	2	175**	120	"	90
713	" " " " "	2	201	124	"	81
714	" " " " "	2	197	142**	"	101**
Average (and S.D.)			201.4 (±13.8)	147.3 (±24.6)	19.2 (±3.7)	89.4 (±7.7)
733	8 per cent NaCl	2	190	124	19.3	81
734	" " " "	2	203	152***	26.8	90
735	" " " "	2	233	138	19.5	81
736	" " " "	2	208	143**	40.4	84
737	" " " "	2	204	146**	41.6	74
738	" " " "	2	241	132	31.0	81
739	" " " "	2	220	140	N.D.	98
740	" " " "	2	213	142**	"	100**
741	" " " "	2	212	142**	"	69
742	" " " "	2	179**	162***	"	88
743	" " " "	2	225	145**	"	98
744	" " " "	2	215	140	"	74
Average (and S.D.)			211.9 (±17.2)	142.2 (±9.4)	29.8 (±9.8)	84.8 (±10.2)

** and ***, differs from control mean value by 2 or 3 S.D. respectively. Both salt-fed groups had higher mean blood pressures and plasma cholesterol concentrations compared with the control group: *blood pressure*, sea salt, $p < 0.05 > 0.01$; NaCl, $p < 0.01$; *cholesterol*, sea salt, $p < 0.01$; NaCl, $p < 0.05 > 0.01$. The mean *weight* of the group fed sea salt was significantly less than the control group: $p < 0.05 > 0.01$.

N.D., not done.

significantly in mean systolic blood pressures, and among individuals with higher pressures no correlation with plasma cholesterol concentration was present. Marked elevations in cholesterol were present in some of the salt-fed animals although among the groups only the mean level in the 2 per cent NaCl animals was significantly higher than that of the control group. The high coefficient of variation of the mean (40 per cent) in the 6 per cent NaCl group presumably accounted for lack of a statistically significant difference from the

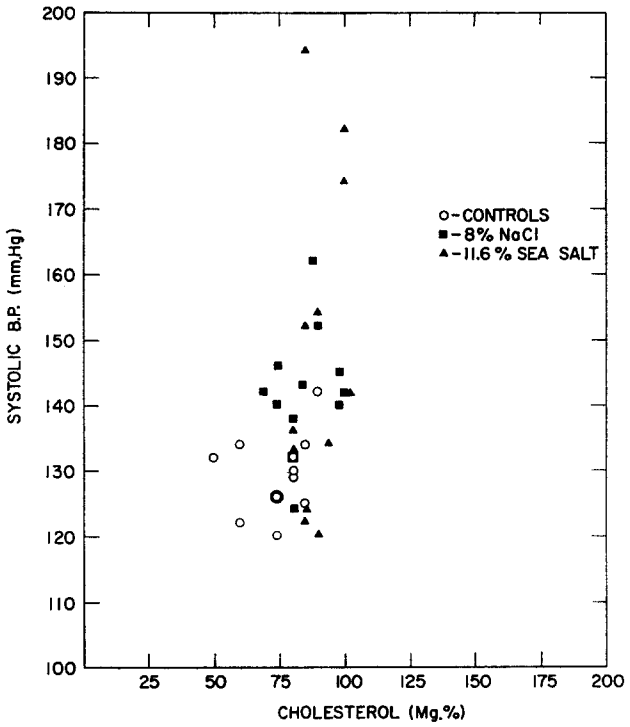

FIG. 3. Effect of chronic salt feeding on blood pressure and plasma cholesterol levels in female rats. Results after 2 months.

mean cholesterol value in the control group; in this 6 per cent NaCl group were two dogs with the greatest elevations in cholesterol namely 386 and 435 mg. per cent. Among the 12 animals fed added salt, 7 had an increase in cholesterol which exceeded 2 standard deviations of the mean value in the control animals, and of these 7, 4 were in excess of 5 standard deviations from this mean. In 8 of the 17 dogs studied, 2 from each group, plasma phospholipids and triglycerides were measured in addition to the cholesterols.[2] These values

[2] The author is indebted to Drs. Howard A. Eder and Lewis Gidez of the Department of Medicine, Albert Einstein College of Medicine, for these determinations.

TABLE IV

17 Beagles Fed Diets Containing Different Amounts of NaCl for 4 Years

Dog	Food	Age	Mos. on diet	Body weight	Systolic B.P.	BUN	Total cholesterol	Phospholipids	Total cholesterol / Phospholipid	Triglycerides
		mos.		*kg.*		*mg. per cent*	*mg. per cent*	*mg. per cent*		*mg. per cent*
1-1	Control	52	50	8.5	160	17.5	153			
8-3	"	52	50	10.6	134	10.3	208			
8-5*	"	52	50	10.2	180	12.2	195	440.0	0.45	18.1
9-3	"	52	50	13.9	146	9.4	177	358.0	0.49	20.4
3-24‡	"	41	40	9.6	171	11.6	186			
Average (and S.D.)				10.6 (±2.0)	158 (±19)	12.2 (±3.2)	184 (±21)			
4-6	6 per cent NaCl	49	45§	7.5	165	9.3	175			
5-2	" " " "	49	45	11.8	145	12.7	386***	618.0	0.63	18.7
5-3	" " " "	49	45	11.0	134	30.4	435***	687.8	0.63	29.3
5-5	" " " "	49	45	16.8	189	11.5	233**			
Average (and S.D.)				11.8 (±3.8)	157 (±24)	16.0 (±9.7)	307 (±123)			
4-8	2 per cent NaCl	50	45	13.4	193	10.8	180			
5-1	" " " "	49	45	11.4	129	12.3	275***			
5-4	" " " "	49	45	13.1	128	10.3	320***	602.5	0.51	70.4
5-6	" " " "	49	45	9.3	139	11.5	290***	476.0	0.61	19.9
Average (and S.D.)				11.8 (±1.9)	147 (±30)	11.2 (±0.9)	266 (±61)			
8-1	1 per cent NaCl	52	48	8.4	135	12.7	193			
8-4	" " " "	52	48	9.1	153	11.2	185			
9-2	" " " "	52	48	14.2	154	12.3	264***	479.0	0.55	17.3
9-5‖	" " " "	52	48	13.6	158	9.1	183	385.2	0.48	11.6
Average (and S.D.)				11.3 (±3.0)	150 (±10)	11.3 (±1.6)	208 (±39)			

All dogs females except No. 9-5 (1 per cent NaCl).

* Dog 8-5 impregnated by No. 9-5 and delivered 2 pups at age 11 months.

‡ Offspring of No. 8-5.

§ First 7½ months, all 6 per cent NaCl dogs received 2 per cent NaCl food, so time on 6 per cent NaCl food was 37½ months.

‖ Male, castrated at age of 9 months.

** and ***, differs from mean control value by 2 or 3 S.D., respectively. Blood pressures not significantly different among groups. Mean cholesterol of 2 per cent NaCl group significantly higher ($p < 0.05 > 0.01$) than control group. The large coefficient of variation (40 per cent) in 6 per cent NaCl group probably contributes to the mean cholesterol level not being significantly greater than that of control group.

and the derived total cholesterol/phospholipid ratio (TC/PL) are shown in the last 3 columns of Table IV. With the same method for phospholipid analysis in another study (10), normal dogs were found to have a serum value of 362 mg. per cent, and a total cholesterol/phospholipid ratio of 0.41. In that work, total cholesterol was analyzed by the Schoenheimer-Sperry method (11), a technique which yields lower values than that of Abell *et al.* used in our study;

therefore the normal TC/PL ratio could be expected to be lower in the earlier study than in the currently reported one. From this very limited sample, there is highly suggestive evidence that other lipids may increase under the same circumstances which lead to an increase in plasma cholesterol in these animals. It is of some interest that animals in the 1 per cent NaCl-H_2O and 6 per cent NaCl food graphs which were ingesting roughly equivalent amounts of NaCl,

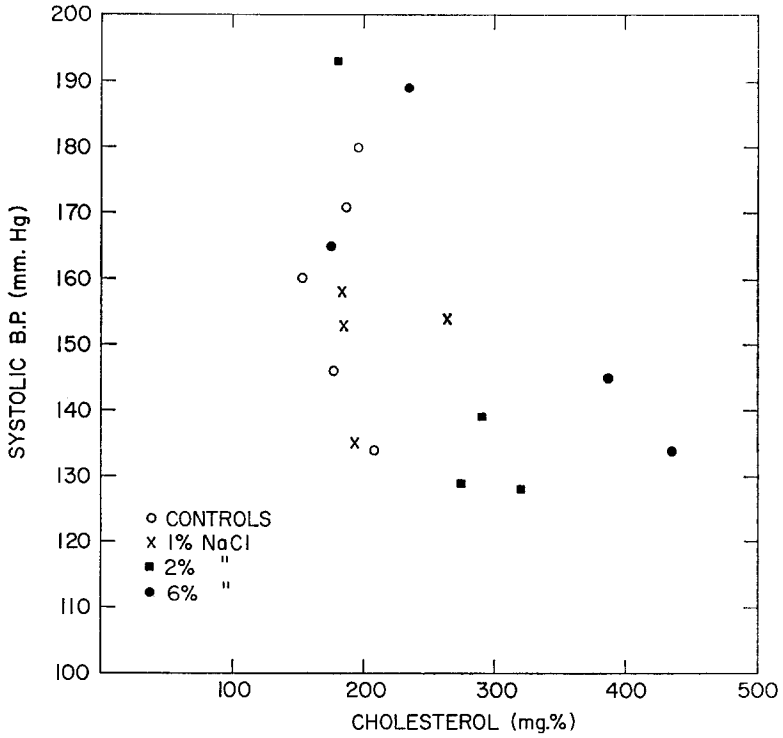

FIG. 4. Effect of chronic salt feeding on blood pressure and plasma cholesterol levels in female dogs. Results after 4 years.

probably differed in the degree to which plasma lipids were increased: the dogs which received salt mainly as a 1 per cent saline drinking solution appeared to respond less dramatically than those which consumed the salt in their food.

DISCUSSION

The present observations indicate that chronic excess salt feeding can be associated with an elevation in plasma cholesterol, and possibly other lipids as well. The data do not suggest that an elevation in blood pressure is necessary

for this phenomenon although they do not rule out the possibility that it may be accentuated by hypertension. It seems clear that chronicity of salt feeding is an important factor in the observation of this abnormality since rats fed extra salt for periods from 2 to 6 months had very modest elevation of plasma cholesterol. In contrast, rats and dogs fed added salt for 15 month and 4 years, respectively, often had quite marked increases in plasma cholesterol concentration.

Dissociation of hypercholesterolemia from elevated blood pressure is made difficult in rats chronically fed excess salt because of the frequency with which salt feeding is followed by some degree of hypertension in these animals. For instance, in one colony of 60 animals now under observation, after 7 months of added salt 78 per cent had hypertension with systolic pressures ranging from 142 to 236 mm. Hg. This means that in such a series, an animal with hypercholesterolemia would have about 4 chances in 5 of also having hypertension, a relatively high correlation but not indicating any necessary causal relationship.

Fortunately, separation of hypertension from hyperlipemia appears to be more definite in the dogs which were fed salt in this study. In these animals, there was no evidence that chronic excess salt feeding resulted in hypertension, possibly because of the great capacity of dogs to excrete oral salt loads rapidly (12). Indeed, among the 7 salt-fed animals with increased plasma cholesterol levels, 5 were non-hypertensive, the 6th was borderline, and the 7th was definitely hypertensive: these last 2 animals also had the lowest cholesterol levels of the 7 with elevations.

Whether hypertension does or can play a primary role in elevating serum lipids is not settled (13). There is evidence derived from animals with experimental hypertension which fails to indicate that hypertension results in elevated serum lipids (14–17). In a study by Lewis, Green, and Page (18), dogs with nephrogenic hypertension had elevated serum lipoprotein values which were thought to be related to kidney damage rather than hypertension: animals with neurogenic hypertension failed to develop these changes. There are several reports of serum lipid concentrations in salt-fed animals which are pertinent. Katz and Stamler (19) produced hypertension in cockerels by the substitution of 0.9 per cent saline solution for drinking water or by the addition of up to 8 per cent sodium chloride to the food and found neither an increase in serum cholesterol or more atherosclerosis as compared with the control chicks. Meneely and coworkers (20–21) have made extensive studies on rats chronically fed excess salt in the manner used in the current studies. They also found a number of animals with elevated serum cholesterols and noted that "serum cholesterol levels tended to increase with increasing dietary sodium chloride, but more striking was the correlation between serum cholesterol and the observed level of the blood pressure. Elevated cholesterol did

not occur in normotensive animals, although not all hypertensive animals were hypercholesterolemic" (21). The summarizing report (21) of their work in which blood pressure and serum cholesterol are correlated, indicates on Fig. VIII that the relationship was similar to that shown in Fig. 1 of the present report. The exceptions are frequent enough to question whether the elevation in pressure was causal to the elevation in cholesterol or not.

Deming *et al.* (22), in studying the effect of experimental hypertension on atherogenesis in the rat, concluded that among animals on an atherogenic diet there was a positive correlation between elevation in blood pressure and serum cholesterol concentration. In attempting to assess the role of salt as distinct from that of desoxycorticosterone they concluded that salt was without effect on plasma lipids. However, the data were not unequivocal on this point: in one set of experiments (Experiment 3) the statement was made that it was "not possible to conclude from these results that salt alone has no effect on cholesterol concentration . . ." . Donomae *et al.* (23) produced experimental atherosclerosis in 52 rabbits by lanolin feeding supplemented in 6 instances with added NaCl: among the 6 animals so treated were those with the highest serum cholesterol in the series. Blood pressures were not measured.

Thus viewed in the fashion indicated, the data from the experiments being reported are not necessarily in discord with earlier experience. There are two considerations which invite speculation: (*a*) what the mechanism of production of this hypercholesterolemia may be and (*b*) whether it plays a role in atherogenesis.

The possible role of the kidney in the elevation of lipids is suggested by the well known nephrotic syndrome in man; Meneely (20–21) reported that some of his salt-fed animals developed a well documented nephrotic-like syndrome. None of the animals in our report presented evidence of such a syndrome at any time. One dog (No. 5-3, Table IV) which had plasma cholesterol concentration of 435 mg. per cent also had a slight elevation of blood urea nitrogen, namely 30.4 mg. per cent; however dog 5-2 in this same group had a normal blood urea nitrogen although the plasma cholesterol was 386 mg. per cent; similarly the 3 rats (Table I) with plasma cholesterols of 380, 400, and 441 mg. per cent respectively, had no nitrogen retention. Nonetheless, more subtle effects on the kidney cannot be ruled out as a cause of this phenomenon.

It seems improbable that the elevated plasma cholesterol concentrations were the result of increased *exogenous* supplies, since the only known variable was salt and this did not influence food consumption. Therefore increases in either intestinal absorption or synthesis of cholesterol or a decrease in the rate of its elimination, are the logical mechanisms which would account for the plasma lipid changes noted here. No data are at hand which would allow a choice from among these possibilities.

It is well established that instillation of water or salt solutions in the gastro-

intestinal tract markedly enhance lymphatic flow (24, 25), and this is a common maneuver in the hands of lymph physiologists. It is intriguing to speculate that these lipid levels might be the result of effects from the chronically increased intestinal lymphatic flow associated with the high salt intake. In this regard it should be recalled that the animals receiving their salt as 1 per cent solution in the drinking fluid had generally lower plasma cholesterol levels than those fed 6 per cent salt with food. The latter consumed about 50 per cent more fluid than did the animals drinking 1 per cent saline. Therefore it is possible that the enhanced fluid intake is the primary factor. It would be interesting to study correlations of uncontrolled diabetes insipidus, clinical or experimental, with hypercholesterolemia.

At the present time, we are aware of no evidence which would suggest that salt plays a role in atherogenesis either in experimental animals or man. Indeed, in the experimental studies of atherosclerosis alluded to above (19, 22, 23) the authors noted specifically that the excess salt ingestion failed to increase the atherosclerotic lesions. Nonetheless, if excessive salt ingestion is capable of elevating plasma lipids it may conceivably affect atherogenesis as well. None of the experimental studies compares in chronicity with the natural history of atherosclerosis in man, in whom slight abnormalities in lipid metabolism operating over decades might result in significant vascular disease.

SUMMARY AND CONCLUSIONS

Among female rats and dogs which were fed excess salt for periods of about 1 and 4 years, respectively, elevations in plasma cholesterol concentrations were frequent and in some cases, quite marked.

Rats on a similar regimen for only 2 and 6 months had less frequent and significantly lesser elevations of cholesterol.

The lipid changes did not appear to be related primarily to increased blood pressure, kidney disease, differences in body weight or food consumption.

There is no present evidence which suggests that this phenomenon is related to atherogenesis although it was speculated that this was possible.

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